two or more CDRs is produced, all that is necessary is to screen the population for species that contain the desired binding affinity modification. All of the criteria for selecting relevant amino acid positions described previously are applicable for use in this mode of the method. Therefore, the methods for modifying or optimizing the binding affinity of a variable region or a heteromeric variable region binding fragment by altering one or more amino acid positions in two or more CDR regions are applicable to essentially any variable region, grafted variable region as well as applicable to the altered and optimized variable regions of the invention.

Moreover, by incorporating variable amino acid residues in two or more CDRs when employing the methods conferring donor CDR binding affinity onto an acceptor framework, this method of modifying binding affinity is therefore useful for simultaneously optimizing the binding affinity of a grafted antibody. Employing the methods for simultaneously grafting and optimizing, or for optimizing, it is possible to generate heteromeric variable region binding fragments having increases in affinities of greater than 5-, 8- and 10-fold. In particular, heteromeric variable region binding fragments can be generated having increases in affinities of greater than 12-, 15- 20- and 25-fold as well as affinities greater than 50-, 100- and 1000-fold compared to the donor or parent molecule.

Additionally, the methods described herein for optimizing are also are applicable for producing catalytic heteromeric variable region fragments or for optimizing their catalytic activity. Catalytic activity can be optimized by changing, for example, the on or off

rate, the substrate binding affinity, the transition state binding affinity, the turnover rate (kcat) or the Km. Methods for measuring these characteristics are well known in the art. Such methods can be employed in the screening steps of the methods described above when used for optimizing the catalytic activity of a heteromeric variable region binding fragment.

The methods for conferring donor CDR binding affinity onto an antibody acceptor variable region framework described previously are applicable for use with essentially any distinguishable donor and acceptor pair. Many applications of the methods will be for the production and optimization of variable region binding fragments having human acceptor frameworks due to the therapeutic importance of such molecules in the treatment of human diseases. However, the method are applicable for conferring donor CDR binding affinity onto an acceptor originating from the same or a divergent species as the CDR donor variable region so long as the framework regions between the donor and acceptor variable regions are distinct. Therefore, the invention included altered variable regions having acceptor frameworks derived from human, mouse, rat, rabbit, goat and chicken, for example.

Additionally, the methods for conferring donor CDR binding affinity onto an antibody acceptor variable region framework are applicable for grafting CDRs as described by Kabat et al., *supra*, Chothia et al., *supra* or MacCallum et al., *supra*. The methods similarly can be used for grafting into an acceptor framework overlapping regions or combinations of CDR as described by these authors. Generally, the methods will graft variable region CDRs by identifying the boundries described by one of the CDR definitions known in the art and set forth

herein. However, because the methods are directed to constructing and screening populations of CDR grafted altered variable regions which incorporate relevant amino acid position changes in both the framework and CDR regions, and such variations can, for example, compensate or augment amino acid changes elsewhere in the variable region, the exact boundry of a particular CDR or set of variable region CDRs can be varied. Therefore, the exact CDR region to graft, whether it is the region described by Kabat et al., Chothia et al. or MacCallum et al., or any combination thereof, will essentially depend on the preference of the user.

Similarly, the methods described previously for optimizing the binding affinity of an antibody also are applicable for use with essentially any variable region for which an encoding nucleic acid is, or can be made available. As with the methods for conferring donor CDR binding affinity, many applications of the methods for optimizing binding affinity will be for modifying the binding affinity of CDR grafted variable regions having human frameworks. Again, such molecules are significantly less antigenic in human patients and therefore therapeutically valuable in the treatment of human diseases. However, the methods of the invention for optimizing the binding affinity of a variable region are applicable to all species of variable regions. Therefore, the invention includes binding affiity optimization of variable regions derived from human, mouse, rat, rabbit, goat and chicken, for example.

The methods of the invention have been described with reference to variable regions and heteromic variable region binding fragments. Given these descriptions and teaching herein, those skilled in the